

STIC-ILL

From: STIC-Biotech/ChemLib
Sent: Wednesday, October 08, 2003 4:27 PM
To: STIC-ILL
Subject: FW: In re: 10.005,510 Journal articles

Mail
only

-----Original Message-----

From: Ford, Vanessa
Sent: Wednesday, October 08, 2003 4:04 PM
To: STIC-Biotech/ChemLib
Subject: In re: 10.005,510 Journal articles

Please supply the following:

SO Poultry Science, (1997) Vol. 76, No. 5, pp. 677-682.

SO VETERINARY RECORD, (16 JAN 1993) Vol. 132, No. 3, pp. 56-59.

SO Journal of Parasitology, (1992) Vol. 78, No. 5, pp. 906-909.

SO AVIAN PATHOL, (1986) 15 (2), 271-278.

SO ACTA PARASITOL POL, (1976 (RECD 1977)) 24 (11-19), 103-117.

Vanessa L. Ford
Biotechnology Patent Examiner
Office: CM1 8A16
Mailbox: CM1 8E12
Phone: 703.308.4735
Art Unit:1645

Papers and Articles

Immunisation of lambs against coccidiosis

J. Catchpole, C. C. Norton, M. W. Gregory

Veterinary Record (1993) 132, 56-59

Two groups of twin lambs, kept with their dams at pasture, were given 10,000 oocysts of *Eimeria crandallis* and 10,000 oocysts of *E ovinoidalis* either at birth only, or on four occasions at weekly intervals. A further group received 1000 oocysts of each species three times a week in a 'trickle infection' from birth to 21 days of age. All these lambs, together with a susceptible control group were challenged with 100,000 oocysts of each species at 28 days of age. A fifth group received no inoculations throughout. Bodyweight, faecal consistency and oocyst output were monitored up to nine weeks of age. There was no clinical response to any of the immunising inoculations and no change in the faecal consistency, but the group infected at birth grew significantly faster than the uninfected controls. The pattern of oocyst output showed that only *E crandallis* developed fully in the newborn animal, but both species multiplied in seven-day-old lambs. The challenge infection produced 80 per cent mortality in the susceptible control group and 20 per cent mortality in the group which had received only one immunising dose at birth. The other immunised groups were well protected and gained more weight than the unchallenged controls. At nine weeks of age, the weight gain of the lambs which had received the 'trickle infection' was significantly higher than that of all the other groups.

COCCIDIOSIS in lambs is a disease associated primarily with intensive husbandry. Of the 11 species of *Eimeria* described in the United Kingdom (Catchpole and others 1975, Norton and Catchpole 1976) *E ovinoidalis* is by far the most pathogenic (Catchpole and others 1976). The second generation meitons and gamonts destroy the crypts in the mucosa of the large intestine, causing a haemorrhagic enteritis (Gregory and Catchpole 1987). Infection with *E crandallis* is often mild, but this species can also produce severe lesions (Catchpole and Gregory 1985); the development stages occur in both the small and the large intestine damaging the crypts and causing a typical greyish scour with a foul odour. The other nine species are not known to be very harmful, but their occurrence in mixed infections can pose problems in diagnosis.

The parasites are ubiquitous. Ewes shed oocysts constantly in small numbers, but the parasite population is increased enormously during the first passages in susceptible lambs. Lambs born later are therefore at greater risk, and stress factors such as multiple births with lower colostrum and milk intake, poor weather, and early weaning exacerbate the condition. Disease occurs in young

lambs between four and eight weeks old, with a peak at six weeks (Gregory and others 1980). Treatment with drugs such as sulphonamides is effective, but because of the rapid onset of clinical signs, the animals suffer a setback in growth. Prevention with anticoccidial compounds in a creep feed is becoming more widely practised, but is dependent upon ear intake by the young lambs when they are primarily suckling.

In less intensive systems disease rarely occurs, because the young lambs meet the parasites more gradually and are able to gain effective immunity.

In previous trials at the Central Veterinary Laboratory, Weybridge, infections with the two pathogenic species have been superimposed upon the natural infections of lambs at pasture.

A heavy single inoculation in lambs up to four days old resulted in no clinical response, whereas inoculation at seven, 14 and 21 days of age caused a loosening of faeces and a slight reduction in weight gain (Gregory and Catchpole 1989). By 28 days of age the animals were more susceptible and showed severe diarrhoea and weight loss. These effects were due mainly to *E ovinoidalis*. *E crandallis* was able to develop in lambs at any age and appeared to elicit a prompt immune response.

Further challenge with 100,000 oocysts of each species at 42 days of age caused severe coccidiosis with 50 per cent mortality in susceptible control animals, while the immunised groups showed only some diarrhoea and weight loss. The effects of challenge were less severe in animals which received later immunising doses at seven, 14 and 21 days (Gregory and Catchpole 1989). Lambs immunised at 28 days old were showing severe clinical signs when they were challenged at 42 days of age, which may have interfered with the establishment of the challenge infection.

In chickens, a 'trickle infection' has been shown to be far more effective in inducing immunity than the administration of the same number of parasites in a single dose (Joyner and Norton 1976, Davis and others 1986). A 'trickle infection' more closely resembles the natural course of infection in the broiler house and in lambs reared extensively at pasture.

The objective of the present investigation was to extend the previous observations by applying immunising doses of the two pathogenic coccidia to young lambs in three different regimens, and measuring their response to a challenge dose.

Materials and methods

Experimental design

A flock of 78 Scottish halfbred and two Hampshire ewes was treated with medroxyprogesterone sponges (Veramix; Upjohn) and pregnant mare's serum to synchronise oestrus and then tupped by Suffolk rams.

The ewes lambred indoors in mid-March and were turned out to pasture after 48 to 72 hours. Only twin lambs were used in the trial; they were randomised into five groups according to birth-



TABLE 1: Experimental design of trial of immunisation of lambs against coccidiosis

| Group | Number of lambs | Immunising dose of equal numbers of oocysts of <i>E crandallis</i> and <i>E ovinoidalis</i> | | |
|-------|-----------------|---|-------------------------------|-----------------|
| | | Dose | Age | Number of doses |
| 1 | 20 | Uninoculated control | | 0 |
| 2 | 18 | 20,000 | At birth | 1 |
| 3 | 20 | 20,000 | At birth, then weekly | 4 |
| 4 | 20 | 2000 | Three times a week from birth | 12 |
| 5 | 18 | Challenge control | | 0 |

Groups 2 to 5 were challenged at four weeks old with 100,000 oocysts of each species

TABLE 2: Mean weight gain of the experimental lambs to 28 days of age expressed as a percentage of birth weight

| Group | Number of lambs | Treatment | Mean (\pm sem) weight gain (% of birth weight) |
|-------|-----------------|----------------------------|---|
| 1+5 | 34 | Uninoculated control | 270.4 \pm 7.2 ^b |
| 2 | 16 | Oocysts at birth | 309.9 \pm 8.8 ^a |
| 3 | 20 | Oocysts weekly | 290.2 \pm 9.8 ^{ab} |
| 4 | 18 | Oocysts three times a week | 299.6 \pm 8.5 ^b |

Values with different superscripts are significantly different P<0.01 (Student's t test).

weight and sex to give 10 pairs of lambs, ie, 20 lambs per group at the start of the trial. All the groups grazed the same area at a stocking density of 20 ewes per hectare. The ewes received a commercial ration for five weeks after lambing but no creep feed was offered to the lambs.

Oocyst suspensions of *E crandallis* and *E ovinoidalis* were prepared from pure cultures of the Weybridge strains passedaged in coccidia-free lambs and were approximately three months old when used in the trial. The inocula were prepared to contain *E crandallis* and *E ovinoidalis* in equal numbers and the lambs were immunised orally, using a ball-ended needle and automatic syringe, according to the regimen outlined in Table 1.

The lambs' bodyweights, faecal consistency and oocyst output were monitored three times a week. The oocysts were counted and the species differentiated as described by MAFF (1986). Faecal consistency was scored 0 for pellets, 1 for unformed pulvaceous faeces, 2 for diarrhoea and 3 for watery or bloody diarrhoea.

Results

Response to immunising injections

Several pairs of lambs had to be withdrawn from the trial either because of injury or because of failure to thrive as a result of poor milk production by the ewe.

All the remaining lambs gained weight steadily and the growth data from birth to 28 days are shown in Table 2. Groups 1 and 5, which received no inoculations up to the age of 28 days, showed comparable weight gains at this stage and are combined into a single group for comparison in Table 2.

The mean weight gain of the immunised groups was greater, and in group 2 the difference was significant (Table 2).

There was no clear pattern to the faecal consistency over this period (Fig 1); most animals passed pellets, but individual lambs occasionally produced a diarrhoeic sample; the incidence was 3.4 per cent, 6.6 per cent and 3.5 per cent in the immunised groups 2, 3 and 4, respectively. The incidence of diarrhoea was 0 and 5.1 per cent for the control groups 1 and 5.

Oocyst output

Background infection with up to eight different species of coccidia picked up from the environment was generally light.

The pattern of oocyst output varied widely in individual ani-

mals, with some producing millions per gram of faeces on the same day as others were negative. The geometric means for each group also had a very wide range of values, so in order to illustrate the trends meaningfully, the geometric means were transformed to the square root and drawn on separate baselines (Figs 2a and 2b).

At the end of its prepatent period, *E crandallis* produced a surge of oocysts. Inoculation at birth produced a light infection with a mean peak output of less than 5000 oocysts/g (opg) peaking at 20 days (Fig 2a). The first two weekly inoculations in group 3 gave mean peak outputs of 8000 and 25,000 opg at 18 and 26 days (Fig 2a). Succeeding inoculations produced no further effect, suggesting that these animals had acquired immunity.

Thrice weekly inoculation of fewer oocysts of *E crandallis* produced a low mean peak output of 8000 opg at 23 days. The numbers quickly declined, indicating that immunity to this infection developed promptly (Fig 2a, group 4).

Background infection with *E crandallis* in the uninoculated controls was light, with mean counts of around 1000 opg, peaking at 26 and 28 days in groups 1 and 5, respectively (Fig 2).

With *E ovinoidalis*, background infection acquired from the pasture was negligible, with mean values less than 400 opg in the uninoculated groups 1 and 5 (Fig 2b) towards the end of the prechallenge period. Similarly only a few oocysts of *E ovinoidalis* were produced by lambs infected at birth (Fig 2b, group 2).

Weekly inoculations in group 3 resulted in a mean peak output of 361,655 opg at 21 days (Fig 2b). The shorter prepatent period of *E ovinoidalis* indicated that these were the result of the second inoculation at seven days of age.

The more gradual acquisition of infection from the inoculation of smaller numbers three times a week reduced the mean output to a shallow peak of 8265 opg at 23 days (Fig 2b, group 4).

Response to the challenge inoculation at 28 days of age

Sixteen of the 20 lambs in group 5, that had received no immunising infection, died or were humanely killed between 14 and 19 days after inoculation, as a result of acute haemorrhagic enteritis (Fig 1). Post mortem examination confirmed that death was due to severe infection with *E ovinoidalis* in the large intestine, coupled with *E crandallis* in the small intestine.

The four lambs which survived were a pair of twins from a Hampshire ewe and two lambs from Scottish half-sired ewes whose twin died. The survivors all showed clinical signs (Fig 1) and produced large numbers of oocysts.

All the lambs in group 2, which had received a single immunising dose of oocysts at birth suffered from bloody diarrhoea. Four animals died (22.2 per cent mortality) between 19 and 23 days after challenge. Post mortem examination confirmed that death was due to acute coccidiosis.

There were no deaths among the lambs which had received multiple immunising doses (groups 3 and 4), nor among the unchallenged control group 1. The lambs' faecal consistency remained normal (Fig 1).

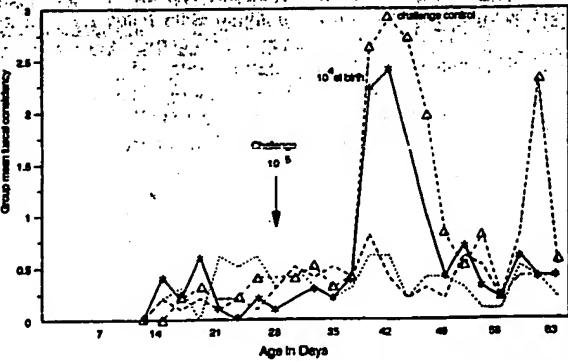


FIG 1: Faecal consistency of the lambs; only two groups showed an adverse reaction to the challenge



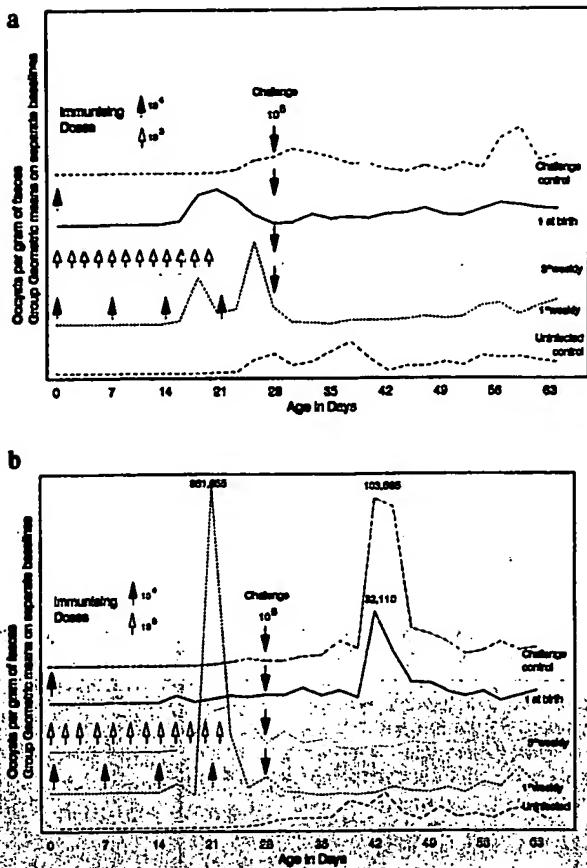


FIG 2: Patterns of oocyst output of *Eimeria crandallis* (a) and *E. ovinoidalis* (b). Group geometric mean oocyst counts transformed to the square root and drawn to the same scale on separate baselines. Certain peak numerical values have been inserted to provide a yardstick.

With the exception of groups 2 and 5, growth proceeded unchecked, the growth rates were analysed at the acute period of the infection 21 days after challenge, just after the deaths had occurred. Growth rates were also analysed 63 days after challenge to observe the longer term effects, and the results are shown in Table 3.

In the group 5 survivors, the lambs from the Hampshire ewe grew unchecked by the infection (Table 3, group 5b) but the Scottish halfbred lambs suffered a severe check in growth despite becoming effectively single lambs on the ewe (Table 3, group 5a).

At the peak of infection resulting from challenge, the lambs which had received immunising oocysts at birth or at weekly intervals (groups 2 and 3) suffered a slight setback in growth and were overtaken by those in group 4 which had received oocysts three times weekly (Table 3, $P<0.05$). None of the weight gains were significantly different from the weight gain of the uninfected control group (Table 3, group 1).

During the next six weeks growth proceeded evenly in all the groups, and by 63 days after challenge the mean weight gains of groups 2 and 3 were very close to the weight gain of the control group (Table 3, group 1). However, the growth of the lambs which had received multiple doses was significantly greater than that of any other group (Table 3, group 4).

Discussion

This experiment demonstrated the value of early infection with pathogenic coccidia in building up resistance to a subsequent heavy challenge.

E ovina ovoidalis, the major pathogen, failed to develop in the new-

TABLE 3: Mean weight gain of the experimental lambs at 21 and 63 days after challenge (expressed as the percentage of weight at challenge at 28 days of age)

| Group | Number of lambs surviving | Treatment | Mean (\pm sem) weight gain (% weight at challenge) | |
|-------|---------------------------|----------------------------|---|------------------------------|
| | | | Days after challenge 21 | 63 |
| 1 | 14 | Uninfected | 140.4 \pm 2.3 ^{a,b} | 232.9 \pm 6.3 ^a |
| 2 | 12 | Oocysts at birth | 134.3 \pm 3.8 ^a | 229.9 \pm 8.0 ^a |
| 3 | 20 | Oocysts weekly | 137.2 \pm 1.6 ^a | 239.6 \pm 4.6 ^a |
| 4 | 18 | Oocysts three times a week | 143.4 \pm 2.2 ^b | 255.2 \pm 4.9 ^b |
| 5a | 2 | Challenge control | 124.5 | 200.5 |
| 5b | 12 | Uninfected control | 147.5 | 274.0 |

Values in each column with different superscripts are significantly different $P<0.05$ (Student's *t* test)

• Surviving Scottish halfbred lambs

† Surviving Hampshire lambs

born animal, possibly because of physiological factors in the large intestine (Gregory and Catchpole 1989). However, in seven-day-old lambs the parasites developed well, as shown by the pattern of oocyst output in group 3 (Fig 2b). When the inoculation at seven days was reinforced by further inoculations at 14 and 21 days, good protection was provided against a heavy challenge at 28 days.

A different pattern of output of *E. ovina* ovoidalis oocysts was seen when the lambs received smaller numbers of oocysts three times a week, a regimen similar to the 'trickle infection' of chickens by Joyner and Norton (1976). These lambs appeared to develop sufficient resistance during the course of the repeated inoculations to reduce the oocyst output to very low levels (Fig 2b, group 4; mean peak output 8265 opg). The subsequent challenge produced no clinical effects and these animals had gained more weight than all the other groups by 63 days (Table 3).

E. crandallis was able to develop in lambs of any age. The first two of the four inoculations at weekly intervals gave rise to distinct peaks of oocyst output (Fig 2a, group 3) whereas the 'trickle infection' produced a single peak with a rapid decline in numbers (Fig 2a, group 4). A similar pattern was seen by Pout and others (1973) in laboratory-reared lambs. None of the immunising infections produced clinical signs (Fig 1), nor did they adversely affect the growth of the animals (Table 2). On the contrary, the weight gains of the immunised groups were greater than the weight gains of the controls (Table 2) and the difference was significant in the lambs inoculated at birth. This phenomenon was observed in earlier trials (Gregory and Catchpole 1989, Gregory and others 1989a) in which a direct comparison was impossible, because the groups of lambs occupied different paddocks. In the present experiment all the animals had common grazing.

The challenge inoculation was administered at 28 days of age so that clinical disease would follow at six weeks, the age at which it most commonly occurs in lambs in the UK (Gregory and others 1980) – and very soon after the peak oocyst output of *E. ovina* ovoidalis and *E. crandallis* from the immunising infections (Figs 2a and 2b). Only *E. crandallis* was able to complete its life cycle in the newborn lamb. *E. ovina* ovoidalis may have partially developed, but no oocysts were produced. These infections induced resistance to a heavy challenge with *E. crandallis* at 28 days, and partial resistance to *E. ovina* ovoidalis. Compared with susceptible controls, the mortality was reduced from 80 per cent to 20 per cent, and the mean peak oocyst output of *E. ovina* ovoidalis was reduced from 103,565 to 32,110 opg (Fig 2b).

The observation that lambs from a Hampshire ewe survived the challenge infection and continued to grow well whereas the lambs from Scottish halfbred ewes died or suffered a severe growth check may indicate a difference in breed susceptibility to coccidial infections. However, only a few lambs were involved and further work is required.

This trial provides further evidence that early challenge with coccidia, before the lamb becomes susceptible to their pathogenic effects, can help to reduce clinical coccidiosis. In many flocks, this process will occur naturally. A trial conducted at Drayton Experimental Husbandry Farm showed clearly that lambs born



under normal conditions, in previously used lambing pens, outperformed those which had been born in clean, previously unused pens (Gregory and others 1989b).

Under normal farming conditions, the deliberate introduction of oocysts in a 'trickle infection' as used in the present trial would be impracticable. However, the results indicated that weekly inoculations also provided good protection; indeed, the more immunising infections the lambs received the better they grew (Table 3) implying that vaccination could improve weight gain even in the absence of heavy environmental contamination. Further research is necessary to arrive at an optimum immunisation regimen. It is unlikely that a live vaccine containing virulent strains of the two pathogenic species would receive licensing approval, but it may prove possible to attenuate them by repeated passage, with selection for precociousness, following the success obtained with *Eimeria* species from chickens (Jeffers 1986) and more recently with *E. intestinalis* from rabbits (Licois and others 1989). Ultimately, the administration of recombinant antigens may provide a more satisfactory means of protecting young lambs from clinical coccidiosis.

Acknowledgements. — The authors thank Jackie Green, Debbie Ferguson and Trudy Jackson for technical assistance with this work. Special thanks are due to Dr Katarzyna Gaca-Lagodzinska, a visitor from Olsztyn, Poland, who assisted with the trial.

References

- CATCHPOLE, J., NORTON, C. C. & JOYNER, L. P. (1975) *British Veterinary Journal* 131, 392
- CATCHPOLE, J. & GREGORY, M. W. (1985) *Parasitology* 91, 45
- CATCHPOLE, J., NORTON, C. C. & JOYNER, L. P. (1976) *Parasitology* 72, 137
- DAVIS, P. J., BARRATT, M. E. J., MORGAN, M. & PARRY, S. H. (1986) Proceedings of Georgia Coccidiosis Conference. Eds L. R. McDougal, L. P. Joyner, P. L. Long. Athens, University of Georgia Press. p618
- GREGORY, M. W. & CATCHPOLE, J. (1987) *International Journal for Parasitology* 17, 1099
- GREGORY, M. W. & CATCHPOLE, J. (1989) *Veterinary Record* 124, 458
- GREGORY, M. W., JOYNER, L. P., CATCHPOLE, J. & NORTON, C. C. (1980) *Veterinary Record* 106, 461
- GREGORY, M. W., CATCHPOLE, J., NOLAN, A. & HEBERT, C. N. (1989a) *Deutsche Tierärztliche Wochenschrift* 96, 287
- GREGORY, M. W., CATCHPOLE, J., JOYNER, L. P. & MAUND, B. A. (1989b) *Veterinary Record* 124, 561
- JEFFERS, T. K. (1986) Proceedings of Georgia Coccidiosis Conference. Eds L. R. McDougal, L. P. Joyner, P. L. Long. Athens, University of Georgia Press. p482
- JOYNER, L. P. & NORTON, C. C. (1976) *Parasitology* 72, 115
- LICOIS, D., COUDERT, P. & BAHAGIA, S. (1989) *Coccidia and Intestinal Coccidiomorphs*, Vth International Coccidiosis Conference, Tours (France), Les Colloques de l'INRA, No 49, p503
- MAFF (1986) Manual of Veterinary Parasitological Laboratory Techniques, Reference book 418. London, HMSO.
- NORTON, C. C. & CATCHPOLE, J. (1976) *Parasitology* 72, 111
- POUT, D. D., NORTON, C. C. & CATCHPOLE, J. (1973) *British Veterinary Journal* 129, 568

Decision support models of leptospirosis in dairy herds

R. M. Bennett

Veterinary Record (1993) 132, 59-61

Following the results of a survey which found that 61 per cent of dairy farmers felt that they needed more information about leptospirosis, and the strategies for its control and the costs and benefits involved, this paper describes the construction and preliminary results of two models of the disease intended to help explore the risks and financial implications of *Leptospira interrogans* serovar *hardjo* infection for dairy producers.

IN a survey of dairy farmers' decisions concerning the control of leptospirosis due to *Leptospira interrogans* serovar *hardjo* infection, 61 per cent of the respondents felt that they needed more or better information about the disease and strategies for its control, together with the costs and benefits involved (Bennett 1991).

Quantitative economic or financial modelling is a formal means of supporting decisions about controlling livestock disease and much has been written about the use of various tools and techniques (Bennett 1992a). In response to the need for information about leptospirosis, this paper describes two computer models which aim to help to explore first, the risks associated with the disease for dairy farmers, and secondly, the financial implications of the disease in dairy herds, so helping to support farmers' decisions on the control of the disease in their herds.

Three main considerations were important when assessing the risks and likely financial implications of the disease to dairy producers: first, the likelihood of a herd suffering from the disease, secondly, the likely effects of the disease on the dairy enterprise, both physically and financially, following the initial infection, for example, of a 'leptospirosis-free' herd, and thirdly, the likely longer term, ie, endemic, effects of the disease in a herd.

The concept of a risk chain (Merkhofer 1987) can be used to consider the risks facing producers from disease in their herds. The risk chain contains four main elements: a hazard, that is, the disease; exposure processes, effects of the hazard and their valuation.

There are several exposure processes which may pose risks of *L. hardjo* affecting a dairy herd. The purchase of infected cattle, co-grazing or common grazing with infected cattle or sheep, the purchase or hire of an infected bull and access of cattle to contaminated water have been identified as important risk factors by Pritchard and others (1989) with further evidence from Bennett (1991). Other factors such as weather conditions and husbandry practices may also be important.

The major physical consequences of *L. hardjo* infection are milk loss and abortion in the dairy herd, and the risk of illness in human beings. Watkins (1986) estimated an overall incidence of around 4 per cent in dairy farm workers, and the disease may also be a cause of infertility in cattle (Ellis 1986). Financial losses for the producer are therefore related mainly to the incidence of abortion and reductions in milk yield and their associated costs. For example, in a study of 50 recently infected herds, Pritchard (1986a) cited abortion rates ranging from 1 to 18 per cent and reductions in milk yield occurred in from 1 to 50 per cent of cows per herd, with a milk loss of around 10 to 30 per cent of expected annual yield in the animals affected (Pritchard 1986b). Assuming an expected annual milk yield of 5000 litres, the cost of a single case of abortion is likely to be at least £366, largely due to the loss of expected milk yield (updated from Bates and others 1984). In contrast, a transient infection causing a reduction in milk yield for a few days is likely to cost no more than £50 per case (assuming a 5 per cent loss in annual expected milk yield at 19.5 pence/litre). The costs associated with disease in people are always difficult to estimate, largely because of the problem of placing a value on human suffering. One tangible cost associated with the disease in a herdsman is the cost of hiring a replacement worker during the period of illness (at around £300 per week) but this is only a partial valuation.

